

FORM PTO-1300 (Modified)  
(REV 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

## TRANSMITTAL LETTER TO THE UNITED STATES

216830US2PCT

DESIGNATED/ELECTED OFFICE (DO/EO/US)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

CONCERNING A FILING UNDER 35 U.S.C. 371

09/926724

INTERNATIONAL APPLICATION NO.

INTERNATIONAL FILING DATE

PRIORITY DATE CLAIMED

PCT/JP00/03697

07 JUNE 2000

08 JUNE 1999

TITLE OF INVENTION

METHOD FOR PREDICTING THE STRUCTURE OF A GENE REGULATOR BINDING SITE AND APPARATUS THEREOF

APPLICANT(S) FOR DO/EO/US

Hiroaki KITANO, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
  - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
  - b. ☒ has been communicated by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
  - a. ☒ is attached hereto.
  - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
  - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
  - b. ☐ have been communicated by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

## Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Notice of Priority / PCT/IB/308 / PTO-1449  
Drawings (7 sheets)

|  |  |  |  |   |  |
|--|--|--|--|---|--|
| U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR<br><b>09/926724</b> |  | INTERNATIONAL APPLICATION NO.<br><b>PCT/JP00/03697</b> |  | ATTORNEY'S DOCKET NUMBER<br><b>216830US2PCT</b> |  |
|--|--|--|--|---|--|

24. The following fees are submitted:

**BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :**

|  |                  |
|--|------------------|
| <input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... | <b>\$1040.00</b> |
| <input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .....  | <b>\$890.00</b>  |
| <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .....  | <b>\$740.00</b>  |
| <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) .....   | <b>\$710.00</b>  |
| <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) .....   | <b>\$100.00</b>  |

**ENTER APPROPRIATE BASIC FEE AMOUNT =**

|  |                 |  |
|--|-----------------|--|
| Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than _____ months from the earliest claimed priority date (37 CFR 1.492 (e)). <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 | <b>\$890.00</b> |  |
|  | <b>\$130.00</b> |  |

| CLAIMS   | NUMBER FILED | NUMBER EXTRA | RATE      |                           |
|--|--------------|--------------|-----------|---------------------------|
| Total claims   | - 20 =       | 0            | x \$18.00 | <b>\$0.00</b>             |
| Independent claims   | - 3 =        | 0            | x \$84.00 | <b>\$0.00</b>             |
| Multiple Dependent Claims (check if applicable) <input type="checkbox"/>   |              |              |           | <b>\$0.00</b>             |
| <b>TOTAL OF ABOVE CALCULATIONS =</b>   |              |              |           | <b>\$1,020.00</b>         |
| <input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.  |              |              |           | <b>\$0.00</b>             |
| <b>SUBTOTAL =</b>  |              |              |           | <b>\$1,020.00</b>         |
| Processing fee of <b>\$130.00</b> for furnishing the English translation later than _____ months from the earliest claimed priority date (37 CFR 1.492 (f)). <input type="checkbox"/> 20 <input type="checkbox"/> 30 + |              |              |           | <b>\$0.00</b>             |
| <b>TOTAL NATIONAL FEE =</b>  |              |              |           | <b>\$1,020.00</b>         |
| Fee for recording the enclosed assignment (37 CFR 1.21 (h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). <input type="checkbox"/>                      |              |              |           | <b>\$0.00</b>             |
| <b>TOTAL FEES ENCLOSED =</b>   |              |              |           | <b>\$1,020.00</b>         |
|  |              |              |           | Amount to be: refunded \$ |
|  |              |              |           | charged \$                |

a. ☒ A check in the amount of **\$1,020.00** to cover the above fees is enclosed.


b. ☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \_\_\_\_\_ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **15-0030**. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:



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*Dec 7, 2001*  
DATE

#4

216830US-2-PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :  
 HIROAKI KITANO ET AL : ATTN: APPLICATION DIVISION  
 SERIAL NO: 09/926,724 :  
 FILED: DECEMBER 7, 2001 :  
 FOR: METHOD FOR PREDICTING THE:  
 STRUCTURE OF A GENE  
 REGULATOR BINDING SITE AND  
 APPARATUS THEREFOR

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS  
 WASHINGTON, D.C. 20231

SIR:

Prior to a first examination on the merits, please amend the above-identified application as follows:

IN THE CLAIMS

Please amend Claim 3 as follows:

3. (Amended) The method for predicting the structure of a gene regulator binding site as described in claim 1, wherein, from the searched parameter sets, microstructures comprising binding sites of the enhancer or promoter, some of the binding sites interacting locally with one another, are predicted as follows:

at portions where binding sites are dense, the microstructures are physically close to one another for interaction therebetween, or they are physically remote from one another but interact with one another closely and functionally, and

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at portions where binding sites are sparse, the microstructures are physically remote from one another and yet functional, or they are functionally independent from one another.

Please add new Claim 6 as follows:

6. (New) The method for predicting the structure of a gene regulator binding site as described in claim 2, wherein, from the searched parameter sets, microstructures comprising binding sites of the enhancer or promoter, some of the binding sites interacting locally with one another, are predicted as follows:

at portions where binding sites are dense, the microstructures are physically close to one another for interaction therebetween, or they are physically remote from one another but interact with one another closely and functionally, and

at portions where binding sites are sparse, the microstructures are physically remote from one another and yet functional, or they are functionally independent from one another.

#### IN THE ABSTRACT

Please amend the Abstract on page 31 as follows:

#### ABSTRACT

A method for predicting the structure of a binding site to which a gene regulator binds, which method enables, through investigation of the internal structure of an enhancer or promoter, transcriptional regulation of a gene to be more clearly elucidated. The method provides a gene of interest, of which a user of the method desires to predict regulation-related structures of binding sites to which regulators bind, the binding sites being present within an enhancer or promoter region to which a protein serving as a transcriptional element is bound and which is present upstream or downstream to a coding region of the gene. A calculation model for each of the binding sites is constructed within the enhancer or promoter region, the

binding sites being such that relevant regulators or hypothetically introduced regulators are to be bound thereto, the calculation model employing, as parameters, loci of the regulator binding sites or other factors that cause expression of the gene. The level of transcription of the gene with respect to the above-constructed calculation model is computed. Through use of parameter search algorithms, parameters of the calculation model are searched so that empirically known expression of the gene is obtained, to thereby predict microstructures of the enhancer or promoter.

#### REMARKS

Favorable consideration of this application, as presently amended, is respectfully requested.

The present Preliminary Amendment is submitted to place the above-identified application in more proper format under United States practice.

By the present Preliminary Amendment the multiple dependency in Claim 3 is cancelled and subject matter of the cancelled multiple dependency is set forth in new dependent Claim 6.

The Abstract has also been amended to be in the form of a single paragraph, to no longer recite any reference numerals, and to make minor idiomatic changes thereto.

The present application is believed to be in condition for a full and thorough examination on the merits. An early and favorable consideration of the present application is hereby respectfully requested.

Respectfully submitted,

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**Marked-Up Copy**  
Serial No: 09/926,724  
Amendment Filed on:

3-19-2002

IN THE CLAIMS

Please amend the claims as follows:

--3. (Amended) The method for predicting the structure of a gene regulator binding site as described in claim 1 [or 2], wherein, from the searched parameter sets, microstructures comprising binding sites of the enhancer or promoter, some of the binding sites interacting locally with one another, are predicted as follows:

at portions where binding sites are dense, the microstructures are physically close to one another for interaction therebetween, or they are physically remote from one another but interact with one another closely and functionally, and

at portions where binding sites are sparse, the microstructures are physically remote from one another and yet functional, or they are functionally independent from one another.--

Claim 6 (New).

IN THE ABSTRACT

Please amend the Abstract on page 31 as follows:

--ABSTRACT

A method for predicting the structure of a binding site to which a gene regulator binds, which method enables, through investigation of the internal structure of an enhancer or

promoter, transcriptional regulation of a gene can be more clearly elucidated. The method [for predicting the gene regulator binding site structure includes providing] provides a gene of interest, of which a user of the method desires to predict regulation-related structures of binding sites [(12), (13), (14)] to which regulators bind, the binding sites [(12), (13), (14)] being present within an enhancer or promoter [(11)] region to which a protein serving as a transcriptional element is bound and which is present upstream or downstream to a coding region [(15)] of the gene[;]. [constructing a] A calculation model for each of the binding sites [(12), (13), (14)] is constructed within the enhancer or promoter [(11)] region, the binding sites [(12), (13), (14)] being such that relevant regulators or hypothetically introduced regulators are to be bound thereto, the calculation model employing, as parameters, loci of the regulator binding sites or other factors that cause expression of the gene[;]. [computing the] The level of transcription of the gene with respect to the above-constructed calculation model[;] is computed. Through [searching, through] use of parameter search algorithms, parameters of the calculation model are searched so that empirically known expression of the gene is obtained[;], to thereby predict microstructures of the enhancer or promoter.--



7/pjs

## Description

METHOD FOR PREDICTING THE STRUCTURE OF A GENE REGULATOR  
BINDING SITE AND APPARATUS THEREFOR

## Technical Field

0926724-014002  
209500-1272600

The present invention relates to regulation of a gene on the basis of, for example, expression data of the gene or concentration data of a protein with respect to the frame of space and time, and in particular to a method for predicting the structure of a binding site to which a gene regulator binds, as well as to an apparatus therefor.

The term "gene" denotes an essential unit of heredity, and encompasses mRNA (messenger ribonucleic acid).

## Background Art

Background art will first be described while Drosophila, which is customarily used in molecular biological studies, is taken as an example.

In the first stage of development of Drosophila, at which point no cells have been formed, transcription products are spread by diffusion. Thus, the concentration of proteins that are present in the vicinity of a certain nucleus plays an important role in regulation of transcription. In simulation of this case, diffusion is calculated according to the following equation:

$$dU_i/dt = D_i \cdot (d^2U_i/dx^2)$$

wherein  $U_i$  is the concentration of a protein  $i$  and  $D_i$  is a

diffusion coefficient of the protein i. Influence of the thus-diffused protein on expression of a gene is calculated by using a probability of binding to the corresponding binding region. For example, for the sake of simplicity, provided that proteins a and b compete for a single binding region, the probability at which the protein a attains binding is calculated as follows:

$$P = U_a / (U_a + U_b)$$

wherein  $U_a$  and  $U_b$  represent concentrations of proteins a and b, respectively.

However, in reality, binding affinity  $\alpha$  must be considered, and therefore, P is expressed as follows.

$$P = \alpha_a \cdot U_a / (\alpha_a \cdot U_a + \alpha_b \cdot U_b)$$

Thus, conventional approaches to understanding of transcriptional regulation of a gene have relied only on protein concentration.

Fig. 1 is a schematic illustration showing conventional transcriptional regulation of a gene.

In Fig. 1, reference numeral 101 represents an enhancer for binding a protein and is located at an upstream region. The enhancer 101 functions as a promoter comprising a switch gene. Reference numeral 102 represents a coding region. Letters A and B represent proteins, and symbols + and - denote activation and repression, respectively.

As described above, transcriptional regulation of a typical gene is generally expressed by an equation in which only the concentration of protein i is employed.

Disclosure of the Invention

However, it has now become clear that the transcriptional regulation of a gene depends not only on the above-mentioned protein concentrations but also on the sites where the proteins are actually bound. Thus, elucidation of the internal structure of an enhancer or promoter has now become a very important subject.

In view of the foregoing, an object of the present invention is to provide, through investigation of the internal structure of an enhancer or promoter and with an aim toward further clarification of transcriptional regulation of a gene, a method for predicting the structure of a binding site to which a gene regulator binds, as well as an apparatus therefor.

To attain the above object, the present invention provides the following:

[1] A method for predicting the structure of a gene regulator binding site, comprising:

a step for providing a gene of interest, of which a user of the method desires to predict the regulation-related structure of a binding site to which a regulator binds, the binding site being present within an enhancer or promoter region to which a protein serving as a transcriptional element is bound and which is present upstream or downstream to a coding region of the gene;

a step for constructing a calculation model for each of

the binding sites within the enhancer or promoter region, the binding sites being such that relevant regulators or hypothetically introduced regulators are to be bound thereto, the calculation model employing, as parameters, loci of the regulator binding sites or other factors that cause expression of the gene;

a step for computing the level of transcription of the gene with respect to the above-constructed calculation model;

a step for searching, through use of parameter search algorithms, parameters of the calculation model so that empirically known expression of the gene is obtained; and

a step for predicting microstructures of the enhancer or promoter.

[2] The method for predicting the structure of a gene regulator binding site as described in [1], wherein said other factors that cause expression of the gene include temperature, molecular weight, diffusion coefficient, binding affinity, reaction rate, reverse reaction rate, and %transcription per occurrence of transcription.

[3] The method for predicting the structure of a gene regulator binding site as described in [1] or [2], wherein, from the searched parameter sets, microstructures comprising binding sites of the enhancer or promoter, some of the binding sites interacting locally with one another, are predicted as follows:

at portions where binding sites are dense, the microstructures are physically close to one another for

interaction therebetween, or they are physically remote from one another but interact with one another closely and functionally, and

at portions where binding sites are sparse, the microstructures are physically remote from one another and yet functional, or they are functionally independent from one another.

[4] Apparatus for predicting the structure of a gene regulator binding site, comprising:

means for providing a gene of interest, of which a user of the method desires to predict the binding site to which a regulator binds, the binding site being present within an enhancer or promoter region to which a protein is bound and which is present in an upstream or downstream region;

means for constructing a calculation model which employs, as parameters, loci of the regulator binding sites within the enhancer or promoter region or other factors that cause expression of the gene;

means for computing the transcription level of the gene with respect to the above-constructed calculation model;

means for searching, through use of parameter search algorithms, parameters of the calculation model so that empirically known expression of the gene is obtained; and

means for predicting microstructures of the enhancer or promoter.

[5] The apparatus for predicting the structure of a gene regulator binding site as described in [4], wherein said

other factors that cause expression of the gene include temperature, molecular weight, diffusion coefficient, binding affinity, reaction rate, reverse reaction rate, and %transcription per occurrence of transcription.

#### Brief Description of the Drawings

Fig. 1 is a schematic illustration showing conventional transcriptional regulation of a gene.

Fig. 2 is a block diagram in relation to an apparatus for predicting the structure of a gene regulator binding site according to an embodiment of the present invention.

Fig. 3 is a flow chart for predicting the structure of a gene regulator binding site and depicting an embodiment of the present invention.

Fig. 4 is a schematic diagram showing exemplary transcriptional regulation of a gene according to the present invention.

Fig. 5 is a conceptual sketch for explaining an enhancer or promoter region of eve2 in relation to an example of the present invention.

Fig. 6 is an enlarged view of the region A shown in Fig. 5.

Fig. 7 shows model structures of binding sites.

Fig. 8 is a graph showing the expression data of eve2.

Fig. 9 is a conceptual illustration of a solution space and solution candidates (candidate solutions) when solution sets are created at random.

Fig. 10 is a conceptual illustration of a solution space and solution candidates when solution sets are not created at random.

Fig. 11 is an illustration showing a solution 1.

Fig. 12 is an illustration showing a solution 2.

Fig. 13 is an illustration showing a solution 3.

Fig. 14 is an illustration showing the case where the binding sites are close to each other.

Fig. 15 is an illustration showing the case where the binding sites are remote from each other.

#### Best Mode for Carrying Out the Invention

Modes for carrying out the present invention will next be described with reference to the accompanying drawings.

Fig. 2 is a block diagram relating to an apparatus for predicting the structure of a binding site of a gene to which a regulator binds according to an embodiment of the present invention.

In Fig. 2, reference numeral 1 indicates an input unit; 2 a processing unit (CPU/memory); 3 a processing section for introducing a gene of interest, of which the user wishes to predict binding sites of regulators within an enhancer or promoter; 4 a processing section for constructing a calculation model in which loci of the binding sites of regulators within an enhancer or promoter are employed as parameters; 5 a processing section for calculating, on the basis of the constructed calculation model, the level of

transcription; 6 a processing section for processing expression pattern data of the gene of interest; 7 a memory section for storing parameter search algorithms; 8 a calculation model parameter search processing section for searching, by use of parameter search algorithms, parameters usable in the calculation model so as to obtain an expression which is in agreement with empirically obtained expression of the aforementioned gene; 9 a processing section for predicting microstructures of an enhancer or promoter; and 10 an output unit.

Fig. 3 is a flow chart for predicting the binding site structure of a gene to which a regulator binds, and depicts an embodiment of the present invention, wherein

(1) First, a gene of interest is provided. The gene is such that there the user of the invention desires to predict the structure of a regulator binding site which is present within an enhancer or promoter which binds a protein and which is present upstream or downstream with respect to a coding region of the gene (Step S1).

(2) Next, a calculation model is constructed for each of the binding sites within the enhancer or promoter region, the binding sites being such that relevant regulators or hypothetically introduced regulators are to be bound thereto, the calculation model employing, as parameters, loci of the regulator binding sites (Step S2).

(3) Subsequently, the level of transcription of the gene is computed with respect to the above-constructed



calculation model (Step 3).

(4) Then, through use of parameter search algorithms, parameters of the calculation model are searched so that empirically known expression of the gene is obtained (Step S4).

(5) Regarding Step S4, checking is performed as to whether parameters cannot be appropriately determined, or whether the obtained results are not good (Step S5).

(6) If the answer to the questions in Step S5 is YES, the constructed model is modified (Step S6).

(7) If the answer to the questions in Step S5 is NO, microstructures of the enhancer or promoter are conjectured (Step S7). Briefly, the microstructures of an enhancer or promoter can be predicted from the parameters obtained by use of search algorithms as follows: at a region where binding sites are dense, the microstructures predictable are such that binding sites are present physically close to one another and closely interact one another, or alternatively they are physically apart from one another but they interact functionally closely one another; and at a region where binding sites are sparse, they act on the gene independently, or in other words, the binding sites exist physically remote from one another, or their actions are functionally independent from one another.

In this connection, when all the regulators cannot be identified, unidentified regulators may be employed as parameters.



(1) Generation of an initial population

A regulatory region of a gene is constructed such that the position of a binding site is used as a variable.

(2) Parameter estimation

For the initial population, parameters are introduced and estimated; the parameter estimation must be effected so as to determine parameters capable of generating expression data which approximate given expression data. The parameter estimation may be performed by use of, for example, a genetic algorithm or an annealing method.

(3) Microstructure estimation

If the above step for parameter estimation reveals, for example, that some regulator binding sites are identified at loci which are close to one another, an inference derived therefrom is that the regulators are also close to one another and interact locally.

On the other hand, if the binding sites are found to be present at loci which are remotely apart from one another, an inference derived therefrom is that the regulators independently and individually act on their respective binding sites.

Hereafter, the method for predicting the structure of a binding site to which a gene regulator binds will be described in detail with reference to Fig. 4.

(1) As shown in Fig. 4, local structures of an enhancer or promoter 11 are introduced (shown as regulator binding sites 12, 13, and 14).

Specifically, the loci corresponding to the respective regulator binding sites 12, 13, and 14 are employed as parameters.

## (2) Parameter optimization

Through optimization of the location of the respective loci, there can be identified microstructures that cannot be functional unless they are close to—or conversely remote from—one another.

To this end, the following procedures are used.

1) Providing a gene of interest, of which the user desires to predict the structures (microstructures) within the enhancer or promoter 11.

2) Constructing a calculation model for each of the binding sites within the enhancer or promoter 11, the binding sites being such that relevant regulators or hypothetically introduced regulators are to be bound thereto. In the calculation model, loci of the regulators are employed as parameters.

3) Computing the level of transcription of the gene with respect to the above-constructed calculation model.

4) Using parameter search algorithms such as GA (genetic algorithm) and SA (simulated annealing), to search parameters of the calculation model so as to obtain empirically known expression of the gene provided in step 1).

5) From the resultant parameter sets, the following are inferred:

(i) At portions where binding sites are dense,

microstructures are physically close to one another, or if they are physically remote from one another, they interact with one another closely and functionally.

(ii) At portions where binding sites are sparse, microstructures are physically remote from one another, or if they are physically close to one another, they are functionally independent from one another.

Next, the method for predicting the structure of a binding site to which a gene regulator binds will be described in more detail.

For illustration purposes, the following description is specifically directed to the case where the gene of interest is the second even-skipped gene (hereinafter referred to as eve2) of Drosophila, which gene relates to the formation of striped patterns.

Fig. 5 is a conceptual sketch for explaining an enhancer or promoter region of eve2 in relation to the exemplary case of the present invention.

In Fig. 5, B1 through B5 are binding sites for a bicoid gene (hereinafter referred to as bcd), H3 for a hunchback gene (hereinafter referred to as hb), K3 through K5 for a Kruppel gene (hereinafter referred to as Kr), and G1 through G3 for a giant gene (hereinafter referred to as gt). The binding sites indicated by circles relate to activation, and those indicated by squares relate to repression. When certain proteins called regulators bind to these binding

sites, the enhancer or promoter activates or represses genetic transcription. Transcription of a gene is considered to be regulated through this mechanism.

As described above, according to most conventional theories, transcriptional regulation of a gene is elucidated through determinism on the basis of concentrations of the respective regulators concerned. For example, in the case of a model which depends on concentration alone, the probability of a transcription of a gene occurring is expressed by the following equation (1).

$$P = \frac{(\alpha_{B4}C_{B4} + \alpha_{B5}C_{B5})}{(\alpha_{B4}C_{B4} + \alpha_{B5}C_{B5} + \alpha_{K5}C_{K5} + \alpha_{G5}C_{G5})}$$

Now, in Fig. 5, attention is invited particularly to the area surrounded by broken lines (region A). Within this region, binding sites of a plurality of regulators are dense on the DNA sequence. Biological studies conducted in recent years have clarified that actual phenomena occurring within such a region A cannot be explained by simple reliance on the concentration of a regulator. In other words, within such a region A, binding and non-binding (which leads to transcriptional regulation of a gene) are determined not on the sole basis of the concentration of a regulator, but also on the basis of local competition in binding caused by high local density of the binding sites, which greatly affects binding and non binding.



Thus, unlike the conventional approach in which concentration alone is used for describing a phenomenon as mentioned above, the method of the present invention, which takes into account not only concentration but also stochastic behavior at different points in time, enables determination of the level of transcription of the gene.

In connection with the aforementioned regulators of eve2, let us assume that the following are known: activating regulators are bcd and hb, and repressing regulators are Kr and gt (or that some of the regulators are unknown and hypothetical introduction thereof is permitted). Under such conditions and in the case where the structure of an enhancer or promoter of eve2 is unknown, or where presence of functional competition/non-competition is unknown, local structures of the regulators can be predicted by performing the below-described steps.

(1) Describing the structure of the enhancer or promoter

The region A in Fig. 5 is enlarged in Fig. 7, where a certain point X is established on the DNA sequence. The distance as counted on the DNA sequence from the point X to the binding site is introduced as a parameter. It is also possible to simultaneously introduce, to each of the binding sites, the size of the binding site itself, binding affinity (or binding probability, dissociation probability, etc.) of the binding site, level of contribution to transcription occurring when a regulator is bound to the binding site, and



other possible variables which may be considered attributes of the binding site.

Specifically, the region A shown in Fig. 5 may be described as follows.

$$\text{Enhancer} \left\{ \begin{array}{l} K5: \text{ distance } a \text{ size } l_a \text{ binding affinity } k_a \dots \\ B5: \text{ distance } b \text{ size } l_b \text{ binding affinity } k_b \dots \\ G3: \text{ distance } c \text{ size } l_c \text{ binding affinity } k_c \dots \\ B4: \text{ distance } d \text{ size } l_d \text{ binding affinity } k_d \dots \end{array} \right. \dots\dots(2)$$

Thus, the introduced binding sites are expressed by means of parameters. By changing the number of binding sites or values of these parameters, generally speaking, any type of enhancer structure or promoter structure can be adequately expressed.

#### (1) Experimental data

During the below-described "parameter search" procedure, experimental data are employed as solutions (also sometimes called optimal values or theoretical values). In the case of the present model, the experimental data are given as follows.

$$\text{Combination } i \left\{ \begin{array}{l} bcd : \alpha_{bcd} < C_{bcd} < \beta_{bcd} \\ hb : \alpha_{hb} < C_{hb} < \beta_{hb} \\ Kr : \alpha_{Kr} < C_{Kr} < \beta_{Kr} \\ gt : \alpha_{gt} < C_{gt} < \beta_{gt} \end{array} \right\} \rightarrow \text{Transcription amount } i \dots\dots(3)$$

$$\text{Combination 2} \left\{ \begin{array}{l} bcd: \alpha_{bcd} < C_{bcd} < \beta_{bcd} \\ hb: \alpha_{hb} < C_{hb} < \beta_{hb} \\ Kr: \alpha_{Kr} < C_{Kr} < \beta_{Kr} \\ gt: \alpha_{gt} < C_{gt} < \beta_{gt} \end{array} \right\} \rightarrow \begin{array}{c} \text{Transcription} \\ \text{Amount 2} \end{array} \dots\dots(4)$$

$$\text{Combination } i \left\{ \begin{array}{l} \text{bod:} \quad \alpha_{bod} < C_{bod} < \beta_{bod} \\ \text{hb:} \quad \alpha_{hb} < C_{hb} < \beta_{hb} \\ \text{Kr:} \quad \alpha_{Kr} < C_{Kr} < \beta_{Kr} \\ \text{gt:} \quad \alpha_{gt} < C_{gt} < \beta_{gt} \end{array} \right\} \rightarrow \begin{array}{c} \text{Transcription} \\ \text{Amount } i \end{array} \dots\dots (5)$$

Briefly, experimental data as to the transcription level of a gene are given in the form of combinations of regulator concentrations as described above. In reality, graphs as shown in Fig. 8 have already become readily available through experiments, and the profiles of the graphs are mathematically expressed by combinations as described above.

### (3) Parameter search

When transcription of the enhancer or promoter expressed as described above is simulated, due to the presence of parameters whose values are not completely determined, the results of simulation may differ from those obtained through experiments. In such a case, through optimization of parameters by use of any appropriate means, parameters for the binding sites that had remained undetermined can be specifically determined.

Next will be described an example drawn to a case in

which GA is employed as a parameter search algorithm which automatically determines parameters of a binding site by use of a computer.

#### [1] Generation of a population

First, a population consisting of a plurality (100, 1,000, or any other plural number) of solution candidates is generated. A solution set is introduced such that parameters are randomly distributed (see Fig. 9), or, for some reason, are converged to a local region of the entire solution space 21 (see Fig. 10). In Fig. 10, reference numeral 22 indicates a subspace in which solutions are conjectured to be present. For example, there may be a solution candidate having a distribution coefficient of 0, or another solution candidate having a distribution coefficient of 1.0. Similarly, there may be a solution candidate having a binding affinity of 0, or another solution candidate having a binding affinity of 0.01. Moreover, regarding the variables such as loci and other items, which have been introduced to the model and are now targets of search, values are changed to thereby provide a plurality of solution candidates.

$$\begin{array}{l} \text{Solution} \\ \text{Candidate 1} \end{array} \left\{ \begin{array}{l} K5 : Da_1 \text{ size } l_{a1} \text{ BA } k_{a1} \dots \\ B5 : Db_1 \text{ size } l_{b1} \text{ BA } k_{b1} \dots \\ G3 : Dc_1 \text{ size } l_{c1} \text{ BA } k_{c1} \dots \\ B4 : Dd_1 \text{ size } l_{d1} \text{ BA } k_{d1} \dots \end{array} \right. \dots\dots(6)$$



information about the transcription level of the gene being handled, the transcription level simulated under the same test environment is compared with the experimentally obtained value. For example, when the transcription level of a gene X is empirically found to be T at a concentration of a regulator A of  $C_A$ , comparison is performed at a fixed concentration  $C_A$  of the regulator A by simulating the transcription level of the gene X and determining the difference between T and the value from the simulation. In simple cases, differences are often represented by absolute values of subtraction (but not necessarily always).

$$\Delta = |T_{\text{simulated}} - T_{\text{found}}| \quad \text{.....(9)}$$

In this case, the smaller the value of  $\Delta$ , the more approximate the simulation to the experimental results. Thus, it can be decided that the solution candidate which can generate a transcription level more approximate to the experimental result is superior to other solution candidates, in order to obtain a transcription level similar to that obtained by experiments.

[4] Artificial selection / natural selection / genetic manipulation

Through comparison with experimental data, the appropriateness of the solution candidate can be assessed. The appropriateness is represented by a "fitness value," and in genetic algorithms, the fitness value represents the

viability of the solution candidate. While solution candidates with excellent fitness values can retry transcription in a subsequent assessment, those with poor fitness values cannot.

In fact, this is the process of artificial selection and natural selection. According to genetic algorithms, after assessment of solution candidates, those with favorable traits are selected, and among the thus-selected candidates assessment is performed again. However, repetition of this process results in reduction in solution candidates, and therefore, replication is performed so as to make the number of candidates constant. Through this process, solution candidates with poor assessment ratings are not selected; and solution candidates which are not selected in subsequent assessment steps are thus screened out.

In genetic manipulations, mutation and cross-over are effected. Specifically, when mutation occurs, a previous diffusion coefficient of 0.5, for example, is changed to 0.6 through a certain value determination method (in some cases randomly changed and in other cases changed through use of a method such as SA). Also, "cross-over" is referred to as a process of selecting a solution candidate A and solution candidate B, and portions of them are mutually exchanged.

Thus through repetition of assessment of fitness values and artificial selection/natural selection/genetic manipulation, the entirety of the solution candidates are gradually refined.

With certain minor errors being ignored, there can be obtained a solution candidate group which has been optimized—or semi-optimized—through an optimization algorithm (note: in the case of optimization being imperfect, the situation may be referred to as "semi-optimized"). From the solution candidate members of the group, actual enhancer or promoter structures can be predicted.

The solutions can be expressed as follows:

$$\text{Optimal Solution } i \left\{ \begin{array}{llll} K5 : D_{a_{opt\ i}} & \text{size } l_{a_{opt\ i}} & BA & k_{a_{opt\ i}} \dots \\ B5 : D_{b_{opt\ i}} & \text{size } l_{b_{opt\ i}} & BA & k_{b_{opt\ i}} \dots \\ G3 : D_{c_{opt\ i}} & \text{size } l_{c_{opt\ i}} & BA & k_{c_{opt\ i}} \dots \\ B4 : D_{d_{opt\ i}} & \text{size } l_{d_{opt\ i}} & BA & k_{d_{opt\ i}} \dots \end{array} \right. \dots\dots(10)$$

wherein D = distance, and BA = binding affinity.

Taking the region A in Fig. 5 as an example, optimization performed as described above may provide optimal solutions as shown in Figs. 11, 12, or 13.

That is, in the case of Fig. 11, a solution candidate provides a situation similar to that shown in region A in Fig. 5.

Alternatively, as in the case of Fig. 12, another solution candidate may provide a similar situation with K5 and G3 being exchanged.

In the case of Fig. 13, yet another solution candidate may provide a situation in which the binding sites are located at discrete positions (loci).

Thus, some converged solution candidates can be obtained.

[5] When solutions are obtained:-

With respect to these solutions, there can be obtained specific values for the enhancer or promoter structures which are desirable for yielding the results mimicking those obtained through experiments. As a result, the following can be detected, speculated upon, or concluded.

(a) In the case where binding sites are densely located, as shown in Fig. 14, since the binding sites are close to each other, these binding sites interact locally during transcription of a gene.

i. There exists a requirement that they must be close to each other as physical loci.

ii. As physical loci they may be remote from each other, but due to their close mutual interaction in terms of function, the solutions are close to each other.

(b) In the case where binding sites are sparsely located, as shown in Fig. 15, since the binding sites are present remote from each other, they independently act on genes during transcription thereof.

i. There exists a requirement that they must be present remote from each other as physical loci.

ii. As physical loci they may be close to each other, but due to their independent functionality, the solutions as obtained result.

Thus, without any information about the structure of an



enhancer or promoter, through simultaneous optimization of values related to binding affinity, reaction rate, reverse reaction rate, etc. of the binding sites, and the situation of interaction of the binding sites contained in the enhancer or promoter, it is possible to know, before actual values are in hand, values which are likely to be found in experiments.

Being constituted as described above, the present invention exerts the following effects.

In the manufacture of chemical substances or pharmaceuticals where the influence of a certain substance on a gene is desired to be investigated in order to know, for example, whether the substance activates or represses the gene, let us assume that the efficacy of the substance acting on the gene is unknown due to the structure of enhancer or promoter of the gene being not completely known. Or alternatively, let us assume that, although speculation is that transcription of the gene is effected under competition between that particular substance and other substances, this speculation has not yet been verified.

Under such circumstances, the present invention enables very prompt and low-cost prediction regarding the efficacy of the substance with respect to its action on the gene, or regarding local interaction of the substance with other substances.

Conventional methods involve drawbacks in that thorough checking of the sequence of target enhancer or promoter structure is required, and the action of unknown substances

cannot be taken into account at all.

However, according to the present invention, since the internal structures of an enhancer or promoter are considered, transcriptional regulation of a gene can be more clearly elucidated. The microstructures of an enhancer or promoter can be predicted from the parameters searched as follows: at a region where binding sites are dense, the loci are present in the vicinity or the binding sites closely interact with one another; and at a region where binding sites are sparse, they act on the gene independently.

Therefore, in cases as described above, the present invention may be implemented as follows: Firstly, computer-aided simulation is performed for prediction, and thereafter, suitable biological experiments are designed and carried out. This process alleviates labor, time, and costs, which have heretofore been a significant burden because of the conventional approaches being performed on a trial and error basis. Moreover, in the pharmaceutical or like industry, prediction of the side effects of a drug can be realized.

The present invention is not limited to the above-described embodiments. Numerous modifications and variations of the present invention are possible in light of the spirit of the present invention, and they are not excluded from the scope of the present invention.

As described hereinabove in detail, according to the present invention, the following effects are obtained.

(A) Through investigation of the internal structure of

an enhancer or promoter, transcriptional regulation of a gene can be more definitely elucidated.

(B) From the parameter set obtained through searching, the microstructures of the enhancer or promoter are predicted such that, in a region where binding sites are dense, the binding sites constitute local microstructures, and in a region where binding sites are sparse, they act on the gene independently.

(C) Thus, in the manufacture of chemical substances or pharmaceuticals, experiments which have conventionally been performed on a trial and error basis, requiring long periods of time, can be performed promptly and accurately.

#### Industrial Applicability

According to the present invention, experiments with respect to regulation of a gene, which have conventionally been performed on a trial and error basis requiring long periods of time can be performed promptly and accurately, and thus the invention is useful in the manufacture of chemical substances and pharmaceuticals.

## Claims

1. A method for predicting the structure of a gene regulator binding site, comprising:

(a) a step for providing a gene of interest, of which a user of the method desires to predict the regulation-related structure of a binding site to which a regulator binds, the binding site being present within an enhancer or promoter region to which a protein serving as a transcriptional element is bound and which is present upstream or downstream to a coding region of the gene;

(b) a step for constructing a calculation model for each of the binding sites within the enhancer or promoter region, the binding sites being such that relevant regulators or hypothetically introduced regulators are to be bound thereto, the calculation model employing, as parameters, loci of the regulator binding sites or other factors that cause expression of the gene;

(c) a step for computing the level of transcription of the gene with respect to the above-constructed calculation model;

(d) a step for searching, through use of parameter search algorithms, parameters of the calculation model so that empirically known expression of the gene is obtained; and

(e) a step for predicting microstructures of the enhancer or promoter.

2. The method for predicting the structure of a gene

regulator binding site as described in claim 1, wherein said other factors that cause expression of the gene include temperature, molecular weight, diffusion coefficient, binding affinity, reaction rate, reverse reaction rate, and transcription per occurrence of transcription.

3. The method for predicting the structure of a gene regulator binding site as described in claim 1 or 2, wherein, from the searched parameter sets, microstructures comprising binding sites of the enhancer or promoter, some of the binding sites interacting locally with one another, are predicted as follows:

at portions where binding sites are dense, the microstructures are physically close to one another for interaction therebetween, or they are physically remote from one another but interact with one another closely and functionally, and

at portions where binding sites are sparse, the microstructures are physically remote from one another and yet functional, or they are functionally independent from one another.

4. Apparatus for predicting the structure of a gene regulator binding site, comprising:

(a) means for providing a gene of interest, of which a user of the method desires to predict the binding site to which a regulator binds, the binding site being present within an enhancer or promoter region to which a protein is bound and which is present in an upstream or downstream

region;

(b) means for constructing a calculation model which employs, as parameters, loci of the regulator binding sites within the enhancer or promoter region or other factors that cause expression of the gene;

(c) means for computing the transcription level of the gene with respect to the above-constructed calculation model;

(d) means for searching, through use of parameter search algorithms, parameters of the calculation model so that empirically known expression of the gene is obtained; and

(e) means for predicting microstructures of the enhancer or promoter.

5. The apparatus for predicting the structure of a gene regulator binding site as described in claim 4, wherein said other factors that cause expression of the gene include temperature, molecular weight, diffusion coefficient, binding affinity, reaction rate, reverse reaction rate, and %transcription per occurrence of transcription.

## Abstract

A method for predicting the structure of a binding site to which a gene regulator binds, which method enables, through investigation of the internal structure of an enhancer or promoter, transcriptional regulation of a gene can be more clearly elucidated.

The method for predicting the gene regulator binding site structure includes providing a gene of interest, of which a user of the method desires to predict regulation-related structures of binding sites (12), (13), (14) to which regulators bind, the binding sites (12), (13), (14) being present within an enhancer or promoter (11) region to which a protein serving as a transcriptional element is bound and which is present upstream or downstream to a coding region (15) of the gene; constructing a calculation model for each of the binding sites (12), (13), (14) within the enhancer or promoter (11) region, the binding sites (12), (13), (14) being such that relevant regulators or hypothetically introduced regulators are to be bound thereto, the calculation model employing, as parameters, loci of the regulator binding sites or other factors that cause expression of the gene; computing the level of transcription of the gene with respect to the above-constructed calculation model; searching, through use of parameter search algorithms, parameters of the calculation model so that empirically known expression of the gene is obtained; to thereby predict microstructures of the enhancer or promoter.

FIG. 1

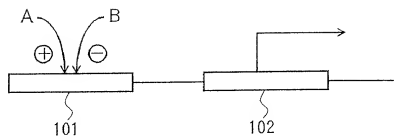




FIG. 2

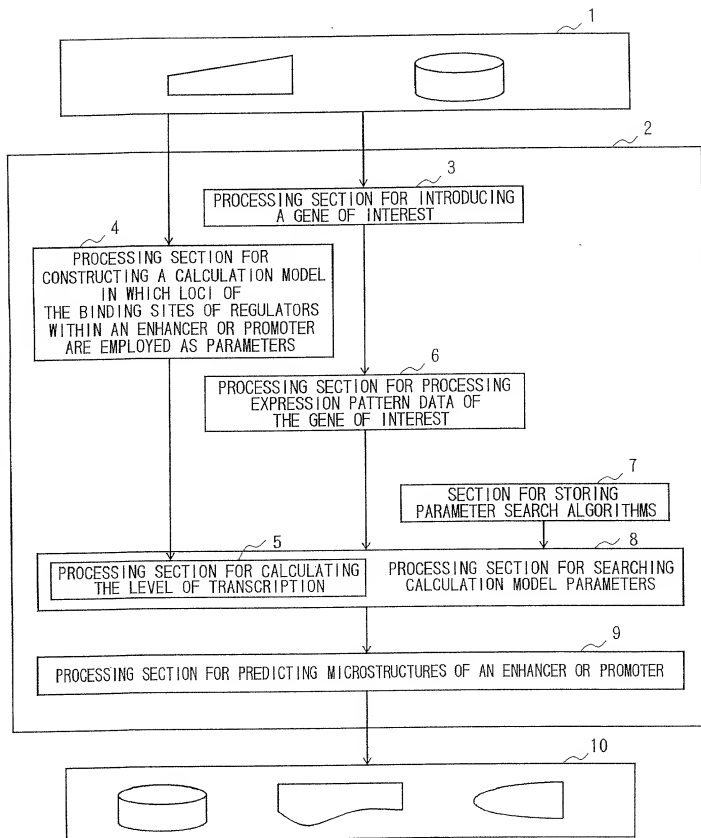


FIG. 3

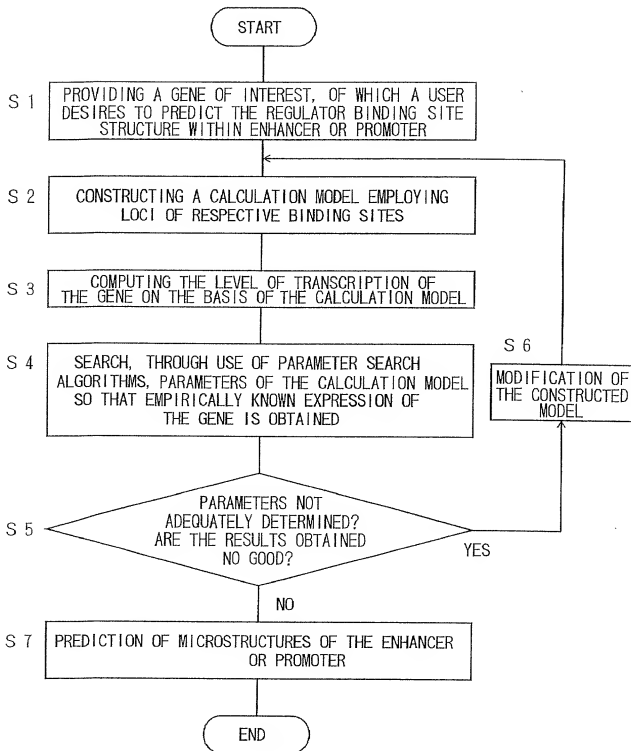


FIG. 4

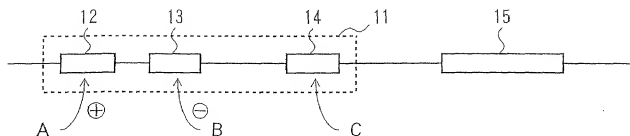


FIG. 5

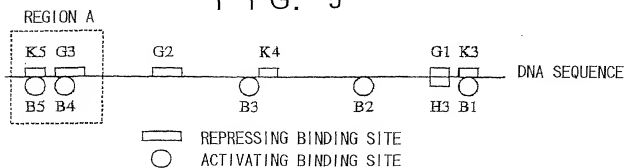


FIG. 6

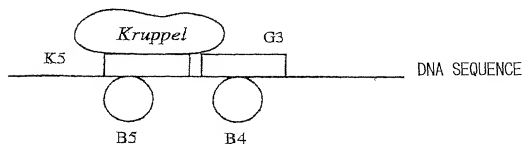


FIG. 7

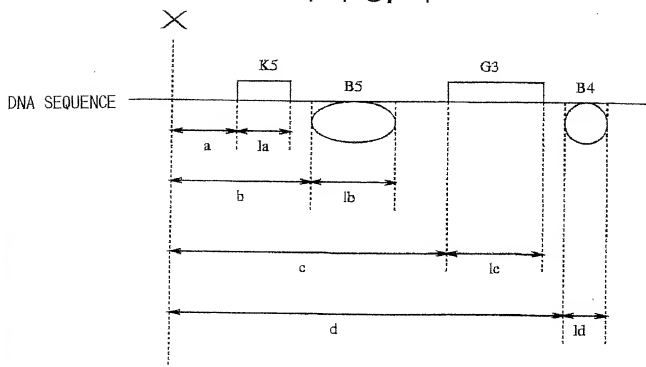


FIG. 8

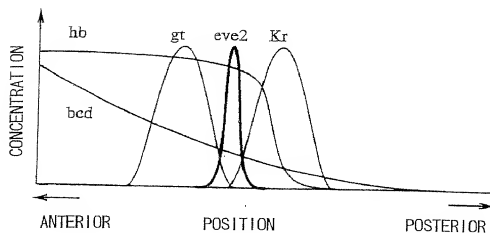


FIG. 9

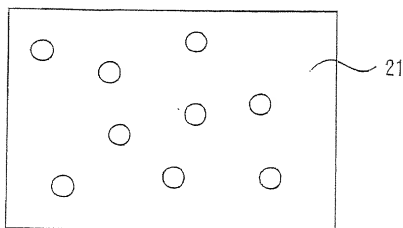


FIG. 10

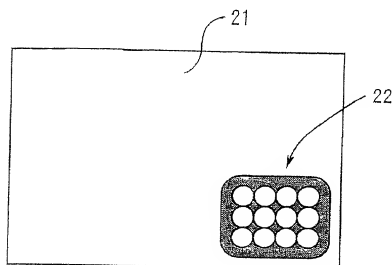


FIG. 11

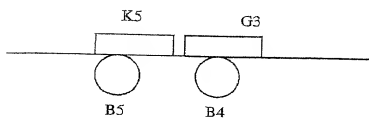


FIG. 12

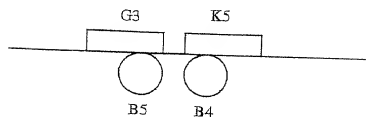


FIG. 13

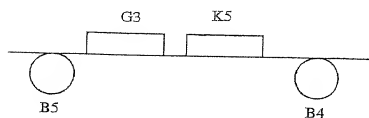


FIG. 14

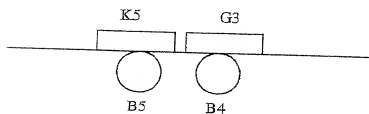
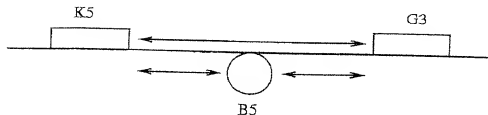


FIG. 15



# Declaration and Power of Attorney For Patent Application

## 特許出願宣言書及び委任状

### Japanese Language Declaration

#### 日本語宣言書

下記の氏名の発明者として、私は以下の通り宣言します。

As a below named inventor, I hereby declare that:

私の住所、私書箱、国籍は下記の私の氏名の後に記載された通りです。

My residence, post office address and citizenship are as stated next to my name.

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者（下記の氏名が一つの場合）もしくは最初かつ共同発明者（下記の名称が複数の場合）であると信じています。

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

METHOD FOR PREDICTING THE STRUCTURE  
OF A GENE REGULATOR BINDING SITE AND  
APPARATUS THEREFOR

the specification of which

☒ is attached hereto.

☒ was filed on June 7, 2000

as United States Application Number or

PCT International Application Number

PCT/JP00/03697 and was amended on  
\_\_\_\_\_ (if applicable).

下記の発明の明細書は、

本書に添付されています。

☐ 月 日 に提出され、米国出願番号または特許協定条  
約国際出願番号を \_\_\_\_\_ とし、  
(該当する場合) \_\_\_\_\_ に訂正されました。

私は、特許請求範囲を含む上記訂正後の明細書を検討し、内容を理解していることをここに表明します。

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

私は、連邦規則法典第37編第1条56項に定義されたとおり、特許資格の有無について重要な情報を開示する義務があることを認めます。

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

**Japanese Language Declaration**  
(日本語宣言書)

私は、米国法典第35編119条 (a) - (d) 項又は365条 (b) 項に基づき下記の、米国以外の国の少なくとも一か国を指定している特許協力条約365 (a) 項に基づく国際出願、又は外国での特許出願もしくは発明者証の出願についての外国優先権をここに主張するとともに、優先権を主張している、本出願の前に出願された特許または発明者証の外国出願を以下に、枠内をマークすることで、示しています。

Prior Foreign Application(s)

外国での先行出願

11 - 160672

**Japan**

(Number)  
(番号)

(Country)  
(国名)

(Number)  
(番号)

(Country)  
(国名)

私は、第35編米国法典119条 (e) 項に基づいて下記の米国特許出願規定に記載された権利をここに主張いたします。

(Application No.)  
(出願番号)

(Filing Date)  
(出願日)

私は、下記の米国法典第35編120条に基づいて下記の米国特許出願に記載された権利、又は米国を指定している特許協力条約365条 (c) に基づく権利をここに主張します。また、本出願の各請求範囲の内容が米国法典第35編112条第1項又は特許協力条約で規定された方法で先行する米国特許出願に開示されていない限り、その先行米国出願書提出日以降で本出願書の日本国内または特許協力条約国際提出日までの期間中に入手された、連邦規則法典第37編1条56項で定義された特許資格の有無に関する重要な情報について開示義務があることを認識しています。

(Application No.)  
(出願番号)

(Filing Date)  
(出願日)

(Application No.)  
(出願番号)

(Filing Date)  
(出願日)

私は、私自信の知識に基づいて本宣言書中で私が行なう表明が真実であり、かつ私の入手した情報と私の信じることに基づく表明が全て真実であると信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第18編第1001条に基づき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行なえば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

I hereby claim foreign priority under Title 35, United States Code, Section 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Priority Claimed

優先権主張

☒ Yes  
はい ☐ No  
いいえ

8/JUNE/1999

(Day/Month/Year Filed)  
(出願年月日)

(Day/Month/Year Filed)  
(出願年月日)

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

(Application No.)  
(出願番号)

(Filing Date)  
(出願日)

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of application.

(Status: Patented, Pending, Abandoned)  
(現況：特許許可済、係属中、放棄済)

(Status: Patented, Pending, Abandoned)  
(現況：特許許可済、係属中、放棄済)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



## Japanese Language Declaration

(日本語宣言書)

委任状：私は下記の発明者として、本出願に関する一切の手続きを米特許商標局に対して遂行する弁理士または代理人として、下記の者を指名いたします。

(弁理士、または代理人の指名及び登録番号を明記のこと)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: (list name and registration number)

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